INTRACELLULAR LOCALIZATION OF MANNAN SYNTHETASE ACTIVITY IN BUDDING BAKER'S YEAST [1]

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SUMMARY: In extracts from budding yeast cells mannan synthetase is present at a much higher activity than in extracts from stationary cells. This activity is largely sedimentable. It is associated with fragments of the plasmalemma, with vesicles known to be involved in the local secretion of glucanases at the site of budding, and with 'light membranes' representing a mixed fraction which probably contains fragments of the endoplasmic reticulum. The possible involvement of these structures in the synthesis and secretion of mannanprotein is discussed.

Mannan synthetase activity is known to be present in particulate preparations from yeast cells (1). Its association with membranes is suggested by the occurrence of a lipophilic mannosyl - intermediate in the transfer reaction from guanosinediphosphate(GDP)-mannose to the final acceptor of mannose residues (2,3,4). The nature of these membranes is, however, not yet known. In a previous investigation on the subcellular distribution of glucanases we presented evidence that in Saccharomyces cerevisiae vesicles containing glucanases are involved in the softening of cell walls at the site of developing buds (5). These vesicles are also characterized by an appreciably high content in mannan. This observation induced us to study the intracellular distribution of mannan synthetase activity.

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Materials and Methods -- Saccharomyces cerevisiae strain LBG H 1022 was cultivated as reported previously (5). The preparation and fractionation of cell free extracts (medium: 0.5 M sorbitol, 0.01 M Tris-HCl buffer pH 7.2, 1mM EDTA) has already been described in detail (5). The sediments 1, 2 and 3 were obtained by differential centrifugation (20 min 20'000 x g; 20 min 55'000 x g; 60 min 150'000 x g). Linear density gradients (4.5 ml) of Urografin[1] extending from 1.08 to 1.20 g cm^{-3} were loaded with 1.0 ml of resuspended particles of 30 min 55'000 x g sediments. After centrifugation for 2.5 hours at 125'000 x g the gradients were divided into 16 fractions. Plasmalemma, glucanase vesicles and light membranes were isolated from several gradients (5), diluted with medium and pelleted. Cell walls were isolated essentially according to (6). The incorporation of radioactivity from guanosinediphosphate-^{14}C-mannose into mannose-polysaccharide was determined employing a reaction mixture (7) containing 20 or 50 µl of enzyme and an equal volume of substrate (4 mM GDP-^{12}C-mannose, 6 mM $MnCl_2$, 0.05 M imidazole-HCl buffer pH 6.5 and GDP-^{14}C-mannose[2] as indicated below). After incubation for 30 or 60 min at 30° carrier mannan (prepared from isolated yeast cell walls) was added and the material insoluble in 60 % ethanol precipitated and washed (7).

Exo-β-glucanase was assayed using p-nitrophenyl-β-glucoside as a substrate (5). Protein was determined in TCA precipitates (8).

Results and Discussion -- The properties of mannan synthetase activity from S. cerevisiae were essentially the same as those

1 Obtained from Schering GmbH, Berlin
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Table 1

Mannan synthetase activity in subcellular fractions obtained from exponentially growing and stationary phase cells

Preparation	^{14}C-mannose incorporated [1] counts / 10 min / mg protein	
	Exponentially growing cells	Stationary phase cells
Total of sedimentable material[2]	1'230	357
Plasmalemma	1'595	1'815
Light membranes	12'950	925

[1] 20 µl of enzyme incubated for 60 min; the substrate contained 20'000 cpm GDP^{14}C-mannose.

[2] 60 min 150'000 x g sediment.

described (7) for the enzyme from S. carlsbergensis. At 30° the incorporation of mannose from GDP-mannose into ethanol insoluble material was practically linear over a 60 min incubation period. Ommission of Mn^{2+} resulted in the reduction of the activity by ca 90 %. The activity was preserved after freezing the enzyme in the presence of 0.5 M sorbitol. Urografin did not interfere with the reaction.

In cell free extracts over 80 % of the total mannan synthetase activity is present in a sedimentable form. The specific sedimentable activity is ca 3.5 times higher in preparations from growing cells as compared with stationary cells (Table 1). In the sediments used for density gradients centrifugation (30 min 55000 x g) ca 65 % of the total mannan synthetase activity was present. In extracts from exponentially growing cells glucanase activity is present in three particulate fractions which, in gradients of

Urografin, equilibrate at different densities. The fraction equilibrating at an intermediate density (ca 1.14 g cm^{-3}) is absent in extracts from stationary cells (5); it consists of small vesicles which are most probably identical with the secretory vesicles observed in developing buds (9). The possible association of mannan synthetase with these vesicles containing mannan in addition to glucanases was examined by comparing the distribution of the activity in density gradients prepared from budding and stationary cells, respectively. It appears from the distribution curves depicted in figure 1 that mannan synthetase as well as exo-β-glucanase are located in three zones of the gradients. In the gradient prepared from budding cells peaks of both enzymes are present in the region of glucanase vesicles, whereas corresponding peaks are absent in the gradients from stationary cells. Vesicles isolated from exponentially growing cells are characterized by the highest specific mannan synthetase activity of all subcellular fractions prepared (Table 2).

Small but significant peaks of mannan synthetase mark the position in the gradients of plasmalemma fragments (10). The activity present in this fraction is independent from the growth conditions as indicated by the almost equal specific activities estimated in plasma membranes isolated from growing and stationary cells (Table 1). A most significant difference between growing and stationary cells concerns the fraction of light membranes (Fig. 1; Table 1). This fraction seems to contribute mainly to the much higher mannan synthetase activity in the budding cell population. The light membrane fraction represents a mixed fraction. It most probably contains the fragments of the endoplasmic reticulum (ER)

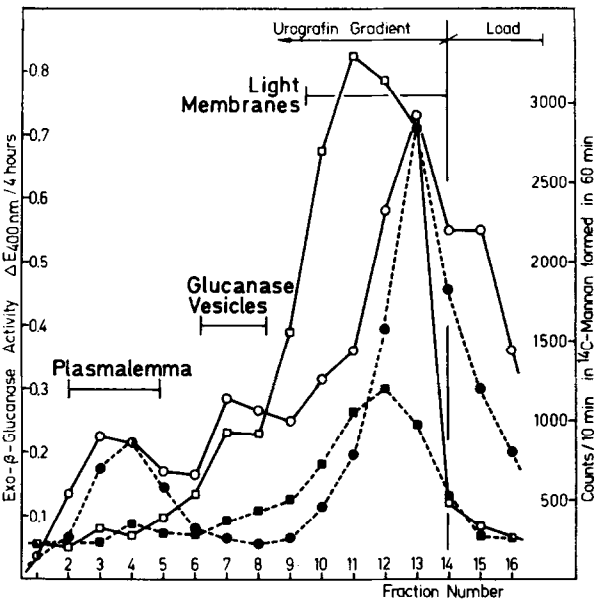

Figure 1 -- Distribution of mannan synthetase and exo-β-glucanase activities present in extracts from growing and stationary cells. 30 min 55'000 x g sediments were used and the activities are corrected for equal amounts of protein (10 mg) loaded onto the gradients. Assay conditions: 50 µl of enzyme were incubated for 4 hrs at 37° (glucanase) and for 60 min at 30° (mannan synthetase). □——□ Mannan synthetase, exponentially growing cells; ■——■ Mannan synthetase, stationary cells; ○——○ Exo-β-glucanase, exponentially growing cells; ●——● Exo-β-glucanase, stationary cells.

as indicated by the presence of typical oxidoreductases (11). We hypothesize that mannan synthetase is a constituent of the ER since morphological observations strongly suggest that the glucanase vesicles represent products of this membrane system (9). In isolated cell walls mannan synthetase activity is virtually absent (Table 2). Hence, synthesis of mannan is likely to be localized in the protoplast exclusively. Yeast spheroplasts are, in fact, known to secrete mannan and mannan containing glycoproteins (12, 13). Glucanase which is also secreted in spheroplasts (14)

may represent one of these glycoproteins. Since cycloheximide inhibits the synthesis of mannan to the same extent as it impairs protein synthesis (15,16), mannan synthesis seems to depend upon the availability of protein moieties of mannanproteins. All of the mannan may, therefore, be exported into the extracellular space in the form of glycoprotein. Hence, it is reasonable to assume that protein moieties of exported mannanproteins are synthesized in the ER, subsequent glycosylation being initiated by mannan synthetase present in this membrane. Upon vesiculation of the ER the synthesis of mannan may continue in the secretory vesicles which, therefore, could be conceived as structures equivalent to

Table 2

Mannan synthetase activity in subcellular fractions obtained from exponentially growing cells

Preparation	µg Protein /50 µl	^{14}C-mannose incorporated [1]	
		counts/10 min	counts/10 min /mg protein
Cell free extract	343	889	2'590
Sediment 1	129	207	1'604
Sediment 2	267	4'898	18'300
Sediment 3	513	1'680	3'270
Soluble fraction	250	314	1'256
Plasmalemma[2]	36	600	16'700
Glucanase vesicles[2]	40	2'088	52'200
Light membranes[2]	46	1'953	42'500
Cell walls	60	-23	0

[1] 50 µl of enzyme incubated for 60 min. The substrate contained 47'200 cpm GDP^{14}C-mannose.

[2] Prepared from sediment 2.

Golgi vesicles. The fusion of vesicles with the plasmalemma results in the secretion of exoenzymes such as the cell wall softening glucanases and of precursor macromolecules of the cell wall mannan. Fusion, in turn, results in the incorporation of the vesicular membranes into the plasmalemma what may explain the presence of mannan synthetase in this membrane. Whether this enzyme has a further function in plasmalemma associated synthesis of mannan is unknown.

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